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Synthesis of a superparamagnetic ultrathin FeCO₃ nanorodsenzyme bionanohybrid as novel heterogeneous catalyst⁺

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Herein we report a straightforward synthesis of ultrathin proteiniron (II) carbonate nanorods (FeCO₃-NRs) heterogeneous bionanohybrid at room temperature and aqueous media. The enzyme induced the *in situ* formation of well-dispersed FeCO₃ NRs on a protein network. The addition of NaBH₄ as reducing agent allowed getting nanowires (5x40 nm) with superparamagnetic properties. This bionanohybrid showed excellent catalytic results in reduction, oxidation and C-C bond reactions.

Iron catalysis has gained an extraordinary attention in the last years in organic chemistry ¹⁻⁴ and the design and development of new types of iron catalysts is highly desirable.

In particular, iron nanostructures with controlled size and composition have been recently developed with different interesting properties.⁵⁻¹⁰ They are of great interest for heterogeneous catalysis because of their unique nanostructure-dependent properties which drastically differentiate their catalytic performance from that of bulk metals.¹¹

Different synthetic methods have been described for iron nanoparticles, where controlling the morphology and size of the nanoparticles exerts tremendous impact on their catalytic properties. ¹²⁻¹⁵ Many of these methods involve the application of hard conditions (e.g. high temperatures or the presence of organic solvents) and the necessity of highly controllable conditions or the utilisation of special equipment. ¹⁴⁻¹⁵

Therefore, the application of new technologies where the iron nanostructures can be synthesized in mild conditions and simple processes where no additional equipment is required for the synthesis of the nanoparticles would represent a tremendous impact. Furthermore, nanorods (NRs) and nanowires (NWs) represent a new class of 1D-nanomaterials which are attracting much attention due to their enhanced optical, magnetic, catalytic and mechanical properties over spherically shaped nanoparticles.¹⁶⁻²² However, only a few examples of these kind of nanostructures of iron (mainly iron oxides) has been reported.²³⁻³⁰ In addition, there are rare cases of the application of iron nanorods or nanowires in catalytic process that have been described.²⁷⁻²⁹

We have developed for the first time the successful design and green synthesis of ultrathin iron (II) carbonate nanorods based on an enzyme (Candida antarctica B lipase) and iron salts (Fig. 1). Well-dispersed nanowires on the biological matrix -avoiding any aggregation problem- were obtained using this methodology at room temperature in aqueous media. The structure of the new heterogeneous nanocatalyst was confirmed by transmission electron microscopy (TEM) combined with other characterization techniques.



Fig. 1. Graphic illustration of the synthesis of $CAL\text{-}B\text{-}FeCO_3$ nanorods bionanohybrid.

Iron (II) carbonate nanorods (FeCO₃NRs) with a diameter of 5-7 nm and length from 40-93 nm were observed by TEM. The iron nanobiohybrid was reduced for 15 min and generated a highly stable and superparamagnetic catalyst, with excellent catalytic efficiency in hydrogenation process in aqueous media, the degradation of aromatic compounds and C-C bond formation.

A similar synthetic approach has been recently applied for designing bionanoconstructs from gold nanoclusters and proteins.³¹⁻³² However, this represents the first time the

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synthesis of magnetic nanorods in a biological environment and with catalytic properties.

The synthesis of this bionanohybrid was performed in aqueous media by adding lipase from Candida antartica B (CAL-B, 33 kDa, monomeric enzyme) to an aqueous solution of fully water soluble iron salts at room temperature and under gentle stirring. Initially (NH4)₂Fe (SO4)₂ (Mohr's salt) was used at different concentrations. 3.6 mL of CAL-B solution (approx. 18 mg protein calculated by Bradford assay³³) was added to 60 mL of iron salt solution at room temperature. In order to control the pH of the final solution -which must be higher than the isoelectric point of the lipase pI=6 to obtain a negatively charged protein and ≤ 10 to avoid iron oxide nanoparticle formation in solution-, different buffers and different concentrations were tested, with 100 mM of sodium bicarbonate (pH 10) the best option. At concentration of 10 mg/mL of iron salts, the solution started to turn cloudy (first step in the bionanohybrid formation) after 30 min with a decrease in the pH of the solution to around 8, which was conserved unaltered during all the incubation time. After 16hours of incubation, a solid was obtained, washed several times with distilled water, centrifuged and lyophilized overnight. ICP-OES analysis revealed that this new bionanohybrid contained 47% (wt.) of iron. The solid was not formed in any case for the enzyme without iron or for the iron salt without protein. XRD and XPS analysis of the solid demonstrated the presence of iron (II) carbonate (siderite, FeCO₃)³⁴ as the main iron species, although some minor contamination of iron oxide (magnetite or maghemite) was found (Figure 2A-B, Figure S1). The agitation speed of the solution in the preparation was an important parameter to avoid the formation of oxidative species (e.g. magnetite). TEM analysis confirmed the formation of nanorods (FeCO₃NRs) with a size of approx. 7 nm in diameter by 59 nm in length induced by the protein matrix, obtaining the so-called CAL-B-FeCO₃NRs-1 bionanohybrid (Figure 2C-D). This hybrid did not possess any evidence of magnetism in the presence of a magnetic field.



Fig. 2.

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Characterization of CAL-B-FeCO₃NRs-1 bionanohybrid.A) XRD. • FeCO₃, * iron oxide impurity. B) XPS. C) TEM. D) HRTEM.

The catalytic activity of this bionanohybrid in the reduction of p-nitrophenol was tested and no conversion was observed.

Therefore, a treatment with sodium borohydride was applied after the previous incubation of the Fe²⁺ salt with CAL-B in bicarbonate buffer. The reduction step was evaluated at different times after adding an aqueous solution of 1.2 M of sodium borohydride (to achieve a final concentration of 0.12 M). After that, the solid was washed three times with water to remove the remaining borohydride, frozen and lyophilized for 18 h to obtain the so called reduced CAL-B-FeCO₃NRs bionanohybrids. When the reduction was performed at 15 min, small nanorods (approx. 5 nm diameter x 40 nm length) as far as we know the smallest reported iron nanorods- were determined by TEM (Figure 3). XRD and XPS demonstrated that $FeCO_3$ was the unique iron species (Figure 3, Figure S2). This reduced bionanohybrid (CAL-B-FeCO₃NRs-2) presented excellent magnetism in the presence of a magnetic field permitting a fast recovery. The analysis of magnetic properties revealed a superparamagnetic material with a saturation magnetization (Ms) value of 125 emu/g considering the total mass of the sample or 267 emu/g considering the iron content of the sample (Figure S3), extremely higher compared with bulk siderite (Ms <0.5 emu/g).



Fig. 3. Characterization of CAL-B-FeCO₃NRs-2 bionanohybrid. A) XRD. B) Full XPS spectrum. C) XPS spectrum of Fe2p. D) TEM/HRTEM.

The reduction was also performed for 30, 45, 60 and 360 minutes recovering the iron bionanohybrids following the same protocol as previously described. The different bionanohybrids were called CAL-B-FeCO₃NRs-3, CAL-B-FeCO₃NRs-4 CAL-B-FeCO₃NRs-5 and CAL-B-FeCO₃NRs-6, respectively. XRD analysis of the different bionanohybrids showed the presence of the same contaminants of Fe(III) observed in CAL-B-FeCO₃NRs-1 which had increasing residual

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amounts in CAL-B-FeCO₃NRs-4 to CAL-B-FeCO₃NRs-6 (Fig S4-S7). TEM analysis revealed the generation of longer-size nanorods, from around 40 to 93 nm, apparently increasing in length according to the reducing time but keeping the same diameter (Table S1, Figures S4-S7).

The activity in the reduction of p-NP by the different reduced CAL-B-FeCO₃NRs bionanohybrids were also tested (Table 1). The reduced bionanohybrid CAL-B-FeCO₃NRs-2 showed high catalytic capacity, being able to complete the total transformation of p-NP to p-AP within 30 seconds (Figure S8).

Table 1. Synthesis of anilines by reduction of nitroarenes using FeCO₃NRs biohybrids as catalysts.^a

	NO ₂		NH ₂	
	FeCO ₃ NRs Biohybrid			
	-	NoDU	≁ [`]	
	R	Distilled water		
		r.t	IX.	
Cataluat	Cubaturata	-	Duradivat	Mi - Lal
Catalyst	Substrate	, ,	Product	Yield
		[sec]		[%]
CAL-B-	NO ₂	360		0
FeCO ₃ NRs-1			$\left[\right]$	
	\checkmark		\checkmark	
	ÓН		ÓН	
	1a		2a	
CAL-B-	1a	30	2a	>99
FeCO ₃ NRs-2				
CAL-B-	1a	45	2a	>99
FeCO ₃ NRs-3				
CAL-B-	1a	45	2a	>99
FeCO₃NRs-4				
CAL-B-	1a	90	2a	>99
FeCO ₂ NRs-5		50		
	15	240	29	92
	10	240	20	52
	NO ₂	120	NH ₂	62
CAL-B-	\checkmark	120	<u> </u>	03
Feco ₃ NRS-2				
	HO' 🗸		HO' ~	
	ID NO-		20	
CAL-B-	HO.	60		72
FeCO ₃ NRs-2	Ϋ́,		Ϋ́,	
	1.		2	
CAL D	NO ₂	000	ZC NH-	- co [b]
CAL-B-	\downarrow	900	\downarrow	>99.1
FeCO ₃ NRS-2				
	~~		\sim	
	он		όн	
	1d		2d	
CAL-B-	NO ₂	300	NH ₂	83 ^[b]
FeCO ₃ NRs-2	\land		\sim	
	Ļ		Ļ	
	Ĭ		Ĭ	
	1e		2e	
CAL-B-	NO ₂	300	NH2	74
	\triangleleft	500	\triangleleft	/4
1 20031413-2	\bigvee		\bigvee	
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	~ 11			

 $^{a}Conditions:$ 1.0 mM (3 mg) nitroarene, 40 mM (3 mg) NaBH_4, 2 mL of distilled water, 3 mg of bionanohybrid, air and room temperature. b 80 mM NaBH_4

A clear correlation between the decrease in the activity of the iron nanocatalyst and the increase in the length of the nanorods with

the presence of the iron oxides contaminant was observed. Around 33% slower rate was obtained for the bionanohybrids reduced for 30 or 45 min in comparison with the results of CAL-B-FeCO₃NRs-2. The CAL-B-FeCO₃NRs-5 bionanohybrid (1h reducing incubation) achieved full reduction after 90 seconds, whereas 95% conversion after 240 seconds was obtained using the CAL-B-FeCO₃NRs-6 (6h reducing incubation).

Thus, these results demonstrated that a short-time reducing step strongly improved the catalytic efficiency of these CAL-B-FeCO₃NRs bionanohybrids for the p-NP degradation where the non-reduced bionanohybrid was not efficient.

The heterogeneous CAL-B-FeCO₃NRs-2 catalyst showed excellent recyclability, conserving more than 95% of its catalytic efficiency in the p-NP reduction after six reaction cycles.

The catalytic activity of this nanobiohybrid was tested with other nitroarenes with high yields in most of cases (Table 1).

One important point for a possible industrial application of this bionanohybrid is the stability against oxidation. The CAL-B-FeCO₃NRs-2 bionanohybrid was stored in aerobic conditions for 30 days. After that time, XRD analysis showed a very small residual impurity of iron oxide in the FeCO₃ spectra (Fig. S9). The catalytic efficiency of the CAL-B-FeCO₃NRs-2 in the reduction of p-NP was still excellent, with a full conversion of the substrate in 45 seconds. TEM analysis revealed the slight increase of the length in the iron NRs which could explain the slight decrease in its efficiency (Fig. S10).

In order to broaden the scope of application of these new iron nanocatalysts, in particular importance the environmental remediation processes, the catalytic capacity of this iron bionanohybrid in the degradation of p-aminophenol (pAP) (hydrolytic product of acetaminophen (paracetamol)) was evaluated. The CAL-B-FeCO₃NRs-2 biohybrid was used to catalyse the reaction in the presence of different hydrogen peroxide concentrations (Figure S11) in aqueous solution at different pHs. The best results were found using 0.5 mM of hydrogen peroxide as an oxidant at room temperature in sodium acetate buffer pH 4 (Figure S11). This nanocatalyst exhibited very good performance under these conditions and more than 98% degradation of pAP was achieved in 2 min (Figure S11). No traces of any compounds were detected by HPLC after 50 min.

The elimination of Bisphenol-A (BPA), an important monomer in the manufacture of plastics, food cans, and other daily-used chemicals, is complex but crucial due to its high toxicity.

Therefore, its degradation to other non or less-toxic products is necessary. However, only a few catalytic processes have been developed. In this way, we tested the application of our nanocatalyst in this process. The CAL-B-FeCO₃NRs-2 biohybrid obtained almost complete degradation of BPA after 3 days using 150 mM of hydrogen peroxide (Figure S12).

Finally, a very interesting application as a magnetic heterogeneous nanocatalyst in C-H functionalization was performed. The Heck reaction between ethylacrylate (**3**) and iodobenzene (**4**) was performed using CAL-B-FeCO₃NRs-2 as a catalyst (entry 3, Table 2). Conversion of 56% producing

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selectively *E*-ethyl cynnamate (5) was achieved at 90°C in dry DMF using trimethylamine as a base in the presence of molecular sieves after 72 h. Also other conditions of varying temperature, base, substrates concentration and additives were tested although none of them improved the results (Table 2, Table S2). Although the total conversion is still to be improved, this result indicates some potential for future applications in this type of reactions.

 Table 2. Heck coupling of aryl iodide with ethyl acrylate catalyzed
by CAL-B-FeCO₃NRs-2 biohybrids as catalyst.



^a3 (0.0274 mmol), 4 (0.055 mmol), 1 mL DMF(dry), 2 equiv NEt₃,5 mg of CAL-B-FeCO₃NRs-2 bionanohybrid.^b 18 mg

In conclusion, we have described a simple, efficient and straightforward methodology to synthesize for the first time an ultrathin iron (ii) carbonate nanorods biohybrid material in multimilligram scale. The strategy allows the synthesize of this nanomaterial at very mild conditions (25°C and atmospheric pressure) without any special equipment using an enzyme to induce the formation of iron (ii) carbonate nanorods in situ on the protein network. This nanobiohybrid was successfully applied as an active and stable superparamagnetic heterogeneous nanocatalyst in nitroarenes reduction, oxidation processes and also suitable for C-H bond functionalization.

These results open the door to extensive application in catalysis.

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Conflicts of interest

There are no conflicts to declare.

Notes and references

- 1) Medicines Agency, Guideline on the Specification Limits for Residues of Metal Catalysts or Metal Reagents, EMEA/ CHMP/SWP/4446/2000, London, February 21, 2008.
- 2) A. Fustner, ACS Cent Sci. 2016, 23, 778.
- 3) B. Plietker, Iron Catalysis in Organic Chemistry, Wiley-VCH, Weinheim, 2008.
- 4) I. Bauer and H.J. Knölker, Chem. Rev., 2015, 115, 3170.

- 5) Y.A. Nor, L. Zhou, A.K. Meka, C. Xu, Y. Niu, H. Zhang, N. Mitter, D. Mahony and C. Yu, Adv. Func. Mat. 2016, 26, 5408
- 6) R. Gao, H. Zhang and D. Yan, Nano Energy, 2017, 31, 90.
- H. Bagheri, E. Ranjbari, M. Amiri-Aref, A. Hajian, Y.H. 7) Ardakani and S. Amidi, Biosen. Bioelec. 2016, 85, 814.
- 8) H. Arami, A. Khandhar, D. Liggitt and K. M. Krishnan, Chem. Soc. Rev. 2015,44, 8576
- 9) N. Lee, D. Yoo, D. Ling, M. H. Cho, T. Hyeon and J. Cheon, Chem. Rev.2015, 115, 10637.
- 10) A. Douglas, R. Carter, L. Oakes, K. Share, A. P. Cohn and C. L. Pint, ACS Nano. 2015, 9, 11156.
- 11) S. Shylesh, V. Schnemann and W. R. Thiel, Angew. Chem. Int. Ed. 2010, 49, 342.
- 12) F. N. Sayed and V. Polshettiwar, Sci. Rep. 2015, 5, 9733.
- 13) V. Polshettiwar and T. Asefa, in Nanocatalysis: Synthesis and Applications (eds. Polshettiwar, V., Asefa, T.) (Wiley, 2013).
- 14) G. Singh, P. A. Kumar, C. Lundgren, A.T. J. van Helvoort, R. Mathieu, E. Wahlström and W. R. Glomm. Part. Part. Svst. Charact. 2014. 31. 1054.
- 15) L.H. Reddy, J.L. Arias, J. Nicolas and P. Couvreur, Chem. Rev. 2012, **112**, 5818.
- 16) D. Lei, J. Benson, A. Magasinski, G. Berdichevsky and G. Yushin, Science, 2017, 355, 267.
- 17) Z. Zhang, M. Sadakane, M. Hara and W. Ueda, Chem. Eur. J. 2017, 23,17497.
- 18) S. Kment, F. Riboni, S. Pausova, L. Wang, H. Han, Z. Hubicka, J. Krysa, P. Schmuki and R. Zboril, Chem. Soc. Rev., 2017, 46, 3716.
- 19) W.Wang, F. Lv, B. Lei, S. Wan, M. Luo and S. Guo, Adv. Mat. 2016. 28.10117.
- 20) J.B. Smith, D. Hagaman, D. DiGuiseppi, R. Schweitzer-Stenner and H.-F. Ji, Angew. Chem. Int. Ed. 2016, 55, 11829.
- 21) S. Zhuo, J. Zhang, Y. Shi, Y. Huang and B. Zhang, Angew. Chem. Int. Ed. 2015, 54, 5693.
- 22) N. Li, P. Zhao and D. Astruc, Angew. Chem. Int. Ed. 2014, 53, 1756
- 23) O. Lupan, V. Postica, N. Wolff, O. Polonskyi, V. Duppel, V. Kaidas, E. Lazari, N. Ababii, F. Faupel, L. Kienle and R. Adelung, Small, 2017, 13.
- 24) S. Shin, S. Kim, J.- Jang and J. Kim, J. Mater. Chem. C, 2017, 5, 1313.
- 25) A.S. Goncharova, S.V. Sotnichuk, A.S. Semisalova, T.Y. Kiseleva, I. Sergueev, M. Herlitschke, K.S. Napolskii and A.A. Eliseev, J. Sol-Gel Scien. Technol, 2017, 81, 327.
- 26) B. Cortés-Llanos, A. Serrano, A. Muñoz-Noval, E. Urones-Garrote, A. Del Campo, J.F. Marco, A. Ayuso-Sacido and L. Pérez, J. Phys. Chem. C, 2017,121, 23158.
- 27) Z.S. Fishman, Y. He, K.R. Yang, A.W. Lounsbury, J. Zhu, T.M. Tran, J.B. Zimmerman, V.S. Batista and L.D. Pfefferle, Nanoscale, 2017,9, 12984.
- 28) D.Xiong, X. Wang, W. Li and L. Liu, Chem.Commun. 2016, 52, 8711.
- 29) S. Das, and S. Jana, Environ. Sci.: Nano, 2017,4, 596.
- 30) C. Zhang, L. Deng, P. Zhang, X. Ren, Y. Li and T. He. Electrochim. Act., 2017, 229, 229.
- 31) S. M. Lystvet, S. Volden, G. Singh, I. M. Rundgren, H. Wen, Ø. Halskauc and W. R. Glomm. J. Phys. Chem. C, 2013, 117, 2230
- 32) S. M. Lystvet, S. Volden, G. Singh, M. Yasuda, Ø. Halskauc and W. R. Glomm, RSC Advances, 2013, 3, 482.
- 33) M.M. Bradford, Anal. Biochem. 1976,72, 248.
- 34) a) X.-F. Qu, Q. I-Z. Yao and G.-T. Zhou, Eur. J. Mineral. 2011, 23, 759; b) W. Xiqing, X. Pengyun, D.Yunfeng, H. Cong and L. Guoping.Inter. J. Min Scien. Technol. 2012, 22, 825; c)RRUFF Project website database: http://rruff.info/Siderite.

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